

## PRIMER NOTE

# Dominant (AFLP) and co-dominant (microsatellite) markers for the kelp *Postelsia palmaeformis* (Laminariales)

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*Department of Ecology and Evolution, The University of Chicago, 1101 East 57th Street, Chicago, IL 60637, USA***Abstract**

To aid in understanding the structure, dispersal and genetic dynamics of their populations, we developed microsatellite and amplified fragment length polymorphism (AFLP) markers for the sea palm, *Postelsia palmaeformis* (Laminariales) for samples taken from nine sites in the area of Cape Flattery, Washington State, USA. We identified two AFLP primers that yielded 798 variable fragments and five microsatellite markers with three to seven alleles each. We also report patterns of allelic variation for four previously identified microsatellite markers in this species and several new alleles.

*Keywords:* AFLP, algae, kelp, microsatellite, *Postelsia palmaeformis*, seaweed

*Received 29 December 2003; revision received 28 January 2003; accepted 2 March 2004*

The sea palm, *Postelsia palmaeformis* (Laminariales), occurs widely on rocky wave-swept shores of the northeast Pacific Ocean from Monterey Bay, California to the northern end of Vancouver Island, British Columbia (Abbott & Hollenberg 1976). In areas where it occurs, it is an extremely productive annual plant (Leigh *et al.* 1987), and it is also harvested in parts of its range, making it an important species to understand ecologically. Furthermore, natural history observations suggest a dual dispersal syndrome, with intact adults apparently releasing spores over very short distances (< 3 m), and detached, reproductive adults rafting for potentially very long distances (Paine 1988, 1979; R. T. Paine personal communication, J.T.W. and C.A.P. unpublished data), which may produce an interesting population structure. Consequently, the development of genetic markers for this species is of interest.

Using minisatellite markers Coyer *et al.* (1997) found extremely high genetic similarity between discrete patches of plants at small scales. Therefore, we were interested in developing finer scale genetic markers to understand the potentially extreme short-distance dispersal in this species. One approach is to use amplified fragment length polymorphism (AFLP; Kusumo & Druehl 2000; see review by Mueller & Wolfenbarger 1999), because it can potentially generate numerous polymorphic markers without prior knowledge of the genome, a common situation for eco-

logically important non-model organisms. However, as a dominant marker system, AFLP suffers from problems in identifying homologous alleles, which is essential for some analytical studies (e.g. heterozygosity analyses; Mueller & Wolfenbarger 1999). Therefore, microsatellite markers were also developed as a potential supplement and methodological check for the AFLP approaches. These markers supplement a set of microsatellite markers developed independently by Whitmer (2002).

DNA was extracted from *P. palmaeformis* tissues collected from nine different sites located 0.4–11 km apart in the vicinity of Cape Flattery, the northwestern tip of the Olympic Peninsula, Washington State USA. At eight of the nine sites we sampled between 18 and 41 individuals while the ninth site was represented by only seven individuals. Fronds from individual live plants were clipped, air-dried, stored in individual aluminium foil packets and express-shipped to the University of Chicago for analysis. As with other kelps, extracting and purifying the DNA of *P. palmaeformis* tissue samples is difficult because of its high mucopolysaccharide content. Several DNA extraction and purification methods were tried; the following three gave consistent results: scaled-down CsCl (Fain *et al.* 1988), CTAB (modified from a protocol of Dr R. Grosberg, University of California, Davis), or REDExtract-N-Amp Plant polymerase chain reaction (PCR) kits (Sigma, catalogue no. XNA-PE). In the modified CTAB protocol, liquid nitrogen-frozen ground tissue was put into a 1.5 mL microtube. We added 600 µL of 2× CTAB solution (0.1 M Tris-HCl pH 8.0, 1.4 M

NaCl, 0.02 M ethylene diaminetetraacetic acid pH 8.0, 2% CTAB, 0.2%  $\beta$ -mercaptoethanol), and incubated the sample at 55 °C for 30 min. Ice-cold chloroform (600  $\mu$ L) was added and the solution was microcentrifuged for 15 min at 16 110 g. The upper layer was transferred to a new microtube. This chloroform purification step was repeated until the interface between the layers was clean. The clean upper layer was then transferred to a new microtube and 600  $\mu$ L 100% ice-cold isopropanol was added. The tube was gently inverted several times to mix the layers. DNA pellets were obtained by spinning the tube for 10 min at 16 110 g and then gently removing the supernatant liquid. The pellet was washed in 70% ethanol, then dried and resuspended in 1 $\times$  TE. Although the scaled-down CsCl and modified CTAB methods worked consistently, we recommend using the REDExtract-N-Amp Plant PCR kit because of its relative simplicity.

The AFLP Plant Genotyping Kit (PE Applied Biosystems) was used. DNA digestion/ligation, pre- and selective amplifications were performed following the manufacturer's suggested protocols, with only a few modifications in the amount of dilutions. After experimentation with various primer pair combinations, four primer pairs were found to consistently amplify *P. palmaeformis* DNA fragments, with the addition of two or three nucleotides on each primer in the selective amplification step. The *Eco*RI primers (E-ACT and E-TG) were labelled with 5-FAM, whereas the *Mse*I primers (M-CAT; M-CAA; M-CAG) were unlabelled. The amplified fragments were detected with an ABI 377 XL automatic sequencer (PE Applied Biosystems) using 36-cm well-to-read Long Ranger 5% polyacrylamide gels (FMC). Fragment sizes and genotypes were analysed using GENESCAN and GENOTYPER (Applied Biosystems) and POPGENE (Yeh &

Boyle 1997) software. To derive criteria for designating peaks in the output, replicate samples were independently extracted and analysed from five plants, and the software was set to optimize the repeatability of samples derived from the same individual. DNA extracts and AFLP profiles from some individuals are available from the authors upon request to facilitate repeatability among researchers.

DNA samples for library construction and microsatellite loci development were isolated by Genetic Identification Services (Chatsworth, CA; <http://www.genetic-id-services.com/>) following a method described by Jones *et al.* (2002). Primers were designed from nine microsatellite-containing sequences using the interactive web interface of PRIMER3 (Rozen & Skaletsky 1998) with the default settings. Forward primers were labelled with fluorescent dye markers NED, 6-FAM or HEX (Applied Biosystems). Reactions were amplified in a PTC-200 Peltier Thermal Cycler (MJ Research) in 10  $\mu$ L reaction volumes with the following temperature profile: incubation of 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 60 °C for 1 min, 72 °C for 45 s, and extension of 72 °C for 10 min. Detection and analysis of amplified DNA fragments followed the protocol for the AFLP above.

AFLP analysis from screening nine populations (a total of 201 individuals) generated an average polymorphism of 61.39% (29.90–77.14%) and 66.58% (25.50–86.00%) at each locus for ACT-CAT primer and TG-CAA primers, respectively.

In our microsatellite analysis, we screened 240 individuals and found that four out of the nine loci identified either were not polymorphic or did not amplify well, despite a variety of efforts to optimize PCR parameters. The remaining five loci were used for subsequent analyses (Table 1); each yielded three to seven alleles. Heterozygosity observed

**Table 1** New microsatellite loci identified in this study, their primer sequences and characteristics based on sample-wide allele frequencies

| Locus†          | Repeat motif                             | Size range | Primers (5'–3')                                     | No. of alleles | $H_E/H_O$        |                  |
|-----------------|--|------------|---|----------------|------------------|------------------|
|                 |  |            |   |                | North Island     | West Rocks       |
| 1<br>(AY286404) | (TAGA) <sub>n</sub>                      | 190–202    | F: CCCACAAACACATTTTCCACA<br>R: ACCAAGGCAACACGTCCTAC | 4              | 0.069/<br>0.000* | 0.307/<br>0.195  |
| 2<br>(AY286405) | (TAGA) <sub>n</sub>                      | 173–193    | F: ATCTTTCACAGCGGCAGGTA<br>R: TTCCCGTGAATTTGGTGTAAA | 4              | 0.231/<br>0.000* | 0.202/<br>0.049* |
| 3<br>(AY286406) | ((CAT) <sub>3</sub> (CAGT)) <sub>n</sub> | 259–328    | F: GGAGCACATCATCACCAGTC<br>R: TGTGTGTTTGGCACACGAAT  | 7              | 0.273/<br>0.209  | 0.202/<br>0.098* |
| 4<br>(AY286407) | (TATC) <sub>n</sub>                      | 217–225    | F: CCGGCATTCAACATAAGCTC<br>R: CTTGGACGAAATGGTTCACA  | 3              | 0.371/<br>0.302  | 0.198/<br>0.073* |
| 5<br>(AY286408) | (ATCT) <sub>n</sub>                      | 222–230    | F: ATAAGGGTCGCTTCTGCTGA<br>R: TCGCGCCTTAGAGAGAAGAC  | 5              | 0.780/<br>0.279* | 0.463/<br>0.000* |

Heterozygosity estimates are provided for two sites, North Island and West Rocks, with  $n = 43$  and  $n = 41$ , respectively; \*denotes deviation from Hardy–Weinberg equilibrium ( $P < 0.05$ ).

†GenBank accession numbers in parentheses.

**Table 2** Analysis of loci reported in Table 1 of Whitmer (2002) screened in samples used in this study

| Locus       | Repeat motif  | Size range | No. of alleles | $H_E/H_O$    |              |
|-------------|---|------------|----------------|--------------|--------------|
|             |   |            |                | North Island | West Rocks   |
| AF501536(6) | (GC(TG) <sub>3</sub> T) <sub>5</sub> (GCCG(TG) <sub>2</sub> T) <sub>6</sub> (GT) <sub>2</sub> GC(TG) <sub>3</sub> T | 225–256    | 3              | —            | — (6)        |
| AF501538(7) | (TG) <sub>8</sub> CG(TG) <sub>4</sub> CGTG(CG) <sub>2</sub> (TGTGCG) <sub>2</sub>                                   | 210–300    | 8              | 0.379/0.047* | 0.072/0.000* |
| AF501539(8) | CA <sub>4</sub> CA <sub>7</sub> (CA) <sub>3</sub> CG(CA) <sub>6</sub> AA(CA) <sub>5</sub> AA(CA) <sub>8</sub>       | 116–223    | 4              | 0.246/0.256  | 0.528/0.195* |
| AF501541(9) | (TC) <sub>20</sub>  | 282–313    | 13             | 0.560/0.279* | 0.793/0.341* |

GenBank accession numbers are given for each locus. Heterozygosity estimates are provided for two sites, North Island and West Rocks, with  $n = 43$  and  $n = 41$ , respectively; \*denotes deviation from Hardy–Weinberg equilibrium ( $P < 0.05$ ). Locus 6 was monomorphic at both sites.

among the five loci tended to be much lower than expected based on Hardy–Weinberg equilibrium (all  $P < 0.001$ , ARLEQUIN Software).

We also tested the microsatellite markers reported by Whitmer (2002) on our samples to evaluate their performance and linkage characteristics (Table 2). Of the six loci she reported, four were polymorphic in our samples, while two were unusable because one produced results with more than two alleles in the same individual, and one did not amplify in our populations. In addition, we found that alleles for all four loci were out of the size range reported by Whitmer (2002) for her populations, and for two loci, we found more alleles than were reported by Whitmer (2002), indicating that we identified a number of new alleles for these microsatellite markers. As Whitmer (2002) found, for the four loci that we applied to our samples, all were significantly less heterozygous than expected at Hardy–Weinberg equilibrium. An analysis of linkage disequilibrium among all nine loci identified eight possible instances of linkage disequilibrium (GENEPOP v. 3.1c; Raymond and Rousset 1995). All loci were involved in at least one of these 16 cases.

We analysed results in detail from the two sites with the highest sampling effort, North Island ( $n = 43$ ) and West Rocks ( $n = 41$ ), to explore marker behaviour at smaller spatial scales (Tables 1 and 2). At North Island and West Rocks, three of eight and one of eight loci were in Hardy–Weinberg equilibrium, respectively, while a ninth locus was monomorphic. Linkage disequilibrium was much less prevalent in this subpopulation analysis, with only one instance at the North Island site (loci AY286408 and AF501541) and none in linkage disequilibrium at West Rocks.

The departure of the microsatellite loci from Hardy–Weinberg equilibrium and the relative deficiency of heterozygotes could be attributed to inbreeding, selection on the loci, a Wahlund effect, or the presence of null alleles. The number of non-amplifying alleles (possible homozygous null individuals) among all individuals and loci was only three out of 2160 (0.14%), a number too small for null alleles to explain the large disparity between expected and observed heterozygosity. Additionally, when we lowered

the annealing temperature for individuals lacking alleles, the results were unchanged, further suggesting that null alleles may not be a problem. In the analysis of two subpopulations, some loci were in Hardy–Weinberg equilibrium and the loci that were not in Hardy–Weinberg equilibrium were inconsistent between populations, implying that the observed deviations were not a result of consistent natural selection. Linkage disequilibrium was also much less prevalent at smaller scales and was inconsistent between the subpopulations. Instead, the general weakening of Hardy–Weinberg equilibrium and linkage disequilibrium patterns and the inconstancy of the patterns between subpopulations are more indicative of a Wahlund effect; population subdivision in this species may be reinforced by limited spore dispersal and the ease with which it self-fertilizes.

Our results suggest that these AFLP and microsatellite markers may be useful for evaluating population structure at small geographical scales in *Postelsia*, and for comparing the relative performance of these two approaches. As a result of the concerns of the repeatability of AFLP analyses, we also plan to compare AFLP and microsatellite performance. A detailed analysis of population differentiation is warranted by our findings and will be presented elsewhere.

### Acknowledgements

We thank the Makah Tribal Council for access to the study sites, the Genetic Identification Services for isolating the microsatellites, and J. Salamunovitch, F. Stevens, K. Rose, B. Kordas, L. Weis, B. Scott, A. Miller, K. Edwards, J. Duke, R. Paine and K. Feldheim for the provision of essential field and laboratory assistance. This work was supported by NSF grant OCE-0117801, University of Chicago Faculty Research Fund, and by the Andrew W. Mellon Foundation.

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